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IS 12895 : 1990

Indian Standard

ALGA *SPIRULINA*, FOOD GRADE — SPECIFICATION

भारतीय मानक

अलगा स्पाइरुलिना, खाद्य ग्रेड — विशिष्टि

UDC 664·38 ALGA

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

August 1990 **Price Group 2**

AMENDMENT NO. 1 JUNE 1991
TO
IS 12895 : 1990 ALGA *SPIRULINA*, FOOD GRADE — SPECIFICATION

[*Page* 1, *Table* 1 , *Sl No.* (iv), *col* 3] — Substitute '55' *for* '5.5'.

(*Page* 2, *clause* A-1.2, *second sentence*) — Substitute the following for the existing sentence:

'A recommended medium is a highly simplified nutrient mix which uses agricultural fertilizer containing nitrogen, phosphorous and potassium in the ratio of 15:15:15 *m/m/m*, magnesium sulphate and sodium bicarbonate.'

(FAD 37)

Reprography Unit, BIS, New Delhi, India

Nutrition Sectional Committee, AFDC 37

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards on 25 March 1990, after the draft finalized by the Nutrition Sectional Committee, had been approved by the Food and Agriculture Division Council.

Use of algal proteins has been suggested for many years as a means to alleviate protein shortage. Since the present availability of milk and other nutritious foods like eggs and meat is low, algal proteins may possibly help in the alleviation of protein calorie malnutrition. *Spirulina*, a blue green alga (cynobacterium), is characterised by high content of protein, β-carotene, B-complex vitamins, particularly cyanocobalamine and gamma-Linolenic acid. The amino acid pattern of this protein shows it to be of reasonably good quality and it may be added to enrich various food and feed formulations. In view of high nutrient content besides proteins, *Spirulina* is marketed Internationally in natural and health foods, in specialised animal feeds and also in cosmetics. This alga has also been approved by FDA of the United States for being used as a natural food. *Spirulina* of species *platensis*, *maxima* and *fusiforms* are at present used for the commercial production of the food grade alga. This standard is therefore being formulated to ensure production of *Spirulina* of certain minimum quality.

Food grade alga *Spirulina* is produced with suitable nutrient media. Though there are variations in the details of the manufacturing processes, the production of food grade alga basically involves the following steps (*see* also Annex A):

a) Selection of suitable strains of alga;
b) Preparation of nutrient medium and specified culture conditions;
c) Cultivation of selected alga in open culture ponds under defined conditions;
d) Separation of grown alga by filtration and washing to get rid of residual nutrients; and
e) Suitable drying to flakes or fine powder.

While formulating this standard due consideration has been given to the relevant rules framed by the Government of India under the Prevention of Food Adulteration Act, 1954 and the Standards of Weights and Measures (Packaged Commodities), Rules 1975. This standard is however subject to the restriction imposed under these wherever applicable

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ALGA *SPIRULINA*, FOOD GRADE — SPECIFICATION

1 SCOPE

1.1 This standard prescribes the requirements and the methods of sampling and tests for food grade alga *Spirulina*.

2 REFERENCES

2.1 The Indian Standards listed in Annex B are necessary adjuncts to this standard.

3 TERMINOLOGY

3.1 Acceptance Tests

Tests carried on samples selected from the lot for the purpose of acceptance of the lot.

3.2 Type Tests

Tests to approve the design and micro nutrient quality of the product at least at the beginning of marketing or certification and conducted periodically thereafter, to check the product quality or whenever the formulation is changed.

4 REQUIREMENTS

4.1 Description

The dried material shall be in the form of powder, flakes or granules. It shall be of uniform dark blue-green colour and shall have the characteristic taste and odour (mild, resembling dried greens) of good quality alga, free from any unpleasant musty or putrid smell. It shall be dry and free from lumps and from extraneous matter, added colour and deleterious substances. It shall also be free from rodent contamination, visible mould growth, insect infestation and toxic materials and heavy metals like lead, copper, mercury and arsenic. Pesticide residues shall be within permissible limits.

NOTE — The appearance, taste and odour of alga shall be determined by sensory tests.

4.2 Hygienic Conditions

The material shall be manufactured in the premises maintained under hygienic conditions.

4.3 The material shall also comply with the requirements given in Tables 1 and 2.

Table 1 Requirements for Alga *Spirulina*, Food Grade

Sl No. (1)	Characteristic (2)	Requirement (3)	Method of Test, Ref to (4)
i)	Moisture, percent by mass, *Max*	9·0	App B of IS 4684 : 1975
ii)	Total ash (on dry basis), percent by mass, *Max*	9·0	App D of IS 4684 : 1975
iii)	Acid insoluble ash (on dry basis), percent by mass, *Max*	0·5	App E of IS 4684 : 1975
iv)	Protein (on dry basis), percent by mass, *Min*	5·5	IS 7219 : 1973
v)	Lead, mg/kg, *Max*	2·5	App J of IS 9808 : 1981
vi)	Arsenic, mg/kg, *Max*	1·1	9 of IS 1699 : 1974
vii)	Cadmium, mg/kg, *Max*	1·0	—
viii)	Mercury, mg/kg, *Max*	0·1	App H of IS 9808 : 1981
ix)	Coliform bacterial count/g, *Max*	Absent (in 0·1 g)	IS 5401 : 1969
x)	*Solmonella* sp.	Absent (in 1 g)	IS 5887 (Part 3) : 1976
xi)	*Shigella*	Absent (in 1 g)	—
xii)	*E. coli*	Absent (in 1 g)	IS 5887 (Part 1) : 1976

Table 2 Vitamins and Mineral Requirement per 100 grams

(*Clause* 4.3)

Sl No. (1)	Characteristic (2)	Requirement (3)	Method of Test, Ref to (4)
i)	Vitamins of the B complex group, mg/100 g	6·0	*See* Note
ii)	Cyanocobalamine (vitamin B_{12}) μg/100 g, *Min*	100	IS 7529 : 1975
iii)	β-carotene, mg/100 g, *Min*	160·0	IS 5886 : 1970
iv)	Phosphorus (P) mg/100 g, *Max*	850·0	IS 6361 : 1971
v)	Iron (Fe), mg/100 g, *Max*	100	App F of IS 1656 : 1985
vi)	Zinc (Zn), mg/100 g, *Max*	35·0	IS 9252 : 1979
vii)	Potassium (K), mg/100 g, *Max*	1350	IS 9497 : 1980

NOTE — The vitamins of the complex group namely thiamine (Vitamin B_1) Riboflavin (Vitamin B_2), Nicotinic acid and Pyridoxine (Vitamin B_6) shall be estimated individually according to the methods given in IS 5398 : 1969, IS 5399 : 1969, IS 5400 : 1969 and IS 7530 : 1975 respectively and their total shall conform to the requirement given under column 3

4.4 Protein Efficiency Ratio (PER)

The food grade alga shall have a corrected PER of not less than 1·7 when determined according to IS 7481 : 1974.

4.5 Other Algal Contamination

The alga culture shall be tested periodically and shall be free from toxic algal forms when tested according to the method given in Annex C.

5 PACKING

5.1 The material shall be packed in clean, sound and suitable containers in such a manner as to prevent absorption of extraneous moisture and prevent undue deterioration during storage.

6 MARKING

6.1 Each container shall be suitably marked to give the following particulars:

a) Name of the material and trade-mark, if any;
b) Indication of the source of manufacture;
c) Date of manufacture;
d) Batch or code number;
e) Nutrients supplied by 100 g;
f) Net mass in g or kg.
g) Any other details required under the Standards of Weights and Measures (Packaged Commodities) Rules, 1977.

7 SAMPLING

7.1 The method of drawing representative samples of the material and the criteria for conformity shall be as prescribed in Annex D.

8 TESTS

8.1 Tests shall be carried out as prescribed in **4.4** and col 4 of Tables 1, 2 and 3.

8.1.1 *Acceptance Tests*

Acceptance tests shall be carried out for the requirements given in Table 1.

8.1.2 *Type Tests*

Type tests shall be carried out for the requirements given in **4.4**, **4.5** and Table 2.

8.2 Unless specified otherwise, pure chemicals shall be employed in tests and distilled water (*see* IS 1070 : 1977) shall be used where the use of water as a reagent is intended.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

ANNEX A
(*Foreword*)

A-0 A recommended process used for the production of food grade algae is given below.

A-1 PROCEDURE

A-1.1 For the purpose of food algae, *Spirulina* of the species *platensis*, *maxima*, and *fusiformis* may be used.

A-1.2 Clean water cultivation of algae is like an agricultural operation and is done in outdoor open tanks made of brick, cement, mortar or PVC material with clean selected strains with specific nutrient medium. A recommended Medium is a highly simplified nutrient mix which uses agricultural fertilizer 'suphala', (N:P:K:15:15:15) Magnesium sulphate and sodium bicarbonate. The culture is agitated and exposed to sunlight and in a week the algal culture blooms when it is harvested. The algal cells are separated from large volumes of water by suitable gravity filters. *Spirulina* being a comparatively large microalgae can be separated on a nylon cloth unlike other algae like *Chlorella* and *Scenedesmus* which can be separated only by centrifugation. Suitable method of drying is very important to obtain alga of good nutritional quality.

ANNEX B
(*Clause* 2.1)

IS No.	*Title*
IS 1070 : 1967	Specification for water for general laboratory use (*second revision*)
IS 1656 : 1985	Specification for milk-cereal based weaning foods (*second revision*)
IS 1699 : 1974	Methods of sampling and test for food colours (*first revision*)
IS 4684 : 1975	Specification for edible groundnut flour (*expeller pressed*) (*first revision*)
IS 4905 : 1968	Methods for random sampling
IS 5398 : 1969	Methods for estimation of thiamine (Vitamin B_1) in foodstuffs
IS 5399 : 1969	Methods for estimation of riboflavin (Vitamin B_2) in foodstuffs

IS No.	*Title*
IS 5400 : 1969	Methods for estimation of nicotinic acid (niacin) in foodstuffs
IS 5401 : 1969	Methods of detection and estimation of coliform bacteria in foodstuffs
IS 5886 : 1970	Methods of estimation of carotenes and vitamin A (Retinol) in foodstuffs
IS 5887 (Part 1) : 1976	Methods for detection of bacteria responsible for food poisoning : Part 1 Isolation, identification and enumeration of *Escherichia Coli* (*first revision*)
IS 5887 (Part 3) : 1976	Methods for detection of bacteria responsible for food poisoning : Part 3 Isolation and identification of *Salmonella* and Shigella (*first revision*)
IS 6361 : 1971	Methods of colorimetric determination of phosphorus
IS 7219 : 1973	Method for determination of protein in foods and feeds
IS 7481 : 1974	Method for determination of protein efficiency ratio (PER)
IS 7529 : 1975	Method of estimation of vitamin B_{12} in foodstuffs
IS 7530 : 1975	Method for estimation of pyridoxine (Vitamin B_6) in foodstuffs
IS 9252 : 1979	Method for determination of zinc (volumetric)
IS 9497 : 1980	Method for determination of sodium and potasium (flame photometric)
IS 9808 : 1981	Specification for fish protein concentrate

ANNEX C

(*Clause* 4.5)

TEST FOR FREEDOM FROM TOXIC ALGAL FORMS

C-1 PROCEDURE

C-1.1 Test Organism Used, *Scenedesmus acutus* (green algae).

C-1.2 Method of Raising Test Culture

Genetically pure strain of *Scenedesmus acutus* maintained as stock culture on agar slants with the medium as given in **C-1.3** and periodically subcultured (every 30 days).

Liquid cultures of *Scenedesmus* is raised from the stock in 250 ml Erlenmeyer flasks in the same medium (each flask to contain 200 ml). The flasks are placed in rotary shaker (30-40 rpm), at temperature (25-28°C) with illumination provided by tubelights 4-5 K lux. The growth rate is monitored as optical density at 560 nm and the algal biomass expressed as mg dry algae/100 ml culture read from standard growth curve (0·1 OD = 100 mg dry algae).

C-1.3 Nutrient Medium

Following standard medium is used for growing *Scenedesmus*.

$(NH_4)_2SO_4$ — 20 mg/l
K_2CO_3 — 25 mg/l
Na_2PO_4 — 25 mg/l
Crude sea salt (NaCl) — 25 mg/l
$MgSO_4 7H_2O$ — 10 mg/l
$FeSO_4$ — 2 mg/l

C-1.4 Testing *Spirulina* Cultures

Cultures are drawn from the culture basins and filtered in 60-80 micron Nylon mesh to remove the *Spirulina* cells and the filtrate is centrifuged at 1 000 rpm for 10 minutes to concentrate other algal forms present in the filtrate. 10 ml of the sedimented filtrate is used in the test for toxicity by adding to the Erlenmeyer flask containing growing *Scenedesmus* cultures at 0·1 OD

Six replicates of control (*Scenedesmus* alone) and experimental (*Scenedesmus* + 10 ml sedimented filtrate) are grown in flasks (**C-1.2**) and growth rate compared for a period of 5 days. Measurements of growth are recorded at OD every day. Reduction of growth of *Scenedesmus* culture by more than 10 percent as compared to control indicates inhibition of algal growth which is attributable to toxic algae being present in cultures.

ANNEX D

(*Clause* 7.1)

SAMPLING OF FOOD GRADE ALGA

D-1 GENERAL REQUIREMENTS OF SAMPLING

D-1.0 In drawing, preparing, storing and handling samples, the following precautions and directions shall be observed:

D-1.1 Samples shall be taken in a protected place not exposed to damp air, dust or soot.

D-1.2 The sampling instrument shall be clean and dry, when used.

D-1.3 Precautions shall be taken to protect the samples, the material being sampled, the sampling instrument and the containers being samples from adventitious contamination.

D-1.4 Samples shall be placed in clean, free from odour and dry glass containers. The containers shall be of such a size that they are almost completely filled by the sample.

D-1.5 Each container shall be sealed air-tight after filling and marked with full details of sampling, batch or code number, name of the manufacturer and other important particulars of the consignment.

D-1.6 Samples shall be stored in such a manner that the temperature of the material does not vary unduly from normal temperature.

D-1.7 Sampling shall be done by a person agreed to between the purchaser and the supplier and in the presence of the purchaser (or his representative) and the supplier (or his representative).

D-2 SCALE OF SAMPLING

D-2.1 Lot

All the containers in a consignment belonging to the same batch of manufacture shall constitute a lot.

D-2.2 Samples shall be tested from each lot for ascertaining conformity of the material to the requirements of this specification.

D-2.3 The number of containers to be tested from a lot shall depend on the size of the lot and shall be in accordance with Table 3.

Table 3 Number of Containers to be Selected for Sampling

Total Number of Containers in the Lot N	Number of Containers to be Selected n
(1)	(2)
Up to 50	3
51 to 200	4
201 to 400	5
401 to 650	6
651 and over	7

D-2.4 These containers shall be chosen at random from the lot. In order to ensure the randomness of selection, procedure given in IS 4905 : 1968 may be followed.

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